

IN THE CLAIMS:

Please cancel claims 423 and 424 without prejudice or disclaimer and add new claims 445 to 483 as shown below.

1-406. (Previously cancelled)

407. (Original) A method of detecting a nucleic acid having at least two portions comprising:

(a) contacting the nucleic acid with a substrate having oligonucleotides attached thereto, the oligonucleotides being located between a pair of electrodes, the oligonucleotides having a sequence complementary to a first portion of the sequence of said nucleic acid, the contacting taking place under conditions effective to allow hybridization of the oligonucleotides on the substrate with said nucleic acid;

(b) contacting said nucleic acid bound to the substrate with a first type of nanoparticles, the nanoparticles being made of a material which can conduct electricity, the nanoparticles having one or more types of oligonucleotides attached thereto, at least one of the types of oligonucleotides having a sequence complementary to a second portion of the sequence of said nucleic acid, the contacting taking place under conditions effective to allow hybridization of the oligonucleotides on the nanoparticles with said nucleic acid; and

(c) detecting a change in conductivity.

408. (Original) The method of Claim 407 wherein the substrate has a plurality of pairs of electrodes located on it in an array to allow for the detection of multiple portions of a single nucleic acid, the detection of multiple different nucleic acids, or both, each of the pairs of electrodes having a type of oligonucleotides attached to the substrate between them.

409. (Original) The method of Claim 407 wherein the nanoparticles are made of metal.

410. (Original) The method of Claim 407 wherein the nanoparticles are made of gold or silver.

411.(Original) The method of Claim 407 wherein the substrate is contacted with silver stain to produce the change in conductivity.

412. (Amended) The method of Claim 407 further comprising:

(d) contacting the first type of nanoparticles bound to the substrate with a second type of nanoparticles, the second type of nanoparticles being made of a material which can conduct electricity, the second type of nanoparticles having one or more types of oligonucleotides attached thereto, at least one of the types of oligonucleotides on the second type of nanoparticles comprising a sequence complementary to the sequence of one of the types of oligonucleotides on the first type of nanoparticles, the contacting taking place under conditions effective to allow hybridization of the oligonucleotides on the first and second types of nanoparticles; and

(e) detecting the change in conductivity.

413. (Original) The method of Claim 412 wherein at least one of the types of oligonucleotides on the first type of nanoparticles has a sequence complementary to the sequence of at least one of the types of oligonucleotides on the second type of nanoparticles and the method further comprises:

(f) contacting the second type of nanoparticles bound to the substrate with the first type of nanoparticles, the contacting taking place under conditions effective to allow hybridization of the oligonucleotides on the first and second types of nanoparticles; and

(g) detecting the change in conductivity.

414. (Original) The method of Claim 413 wherein step (d) or steps (d) and (f) are repeated one or more times and the change in conductivity is detected.

415. (Amended) The method of Claim 407 further comprising:

(d) contacting the first type of nanoparticles bound to the substrate with an aggregate probe comprising a second type of nanoparticles having one or more types of oligonucleotides attached thereto, the nanoparticles of the aggregate probe being made of a material which can conduct electricity, at least one of the types of oligonucleotides on the

aggregate probe comprising a sequence complementary to the sequence of one of the types of oligonucleotides on the first type of nanoparticles, the contacting taking place under conditions effective to allow hybridization of the oligonucleotides on the aggregate probe with the oligonucleotides on the first type of nanoparticles;

(e) and detecting the change in conductivity.

416. (Previously amended) A method of detecting nucleic acid having at least two portions comprising:

(a) contacting a nucleic acid with a substrate having oligonucleotides attached thereto, the oligonucleotides being located between a pair of electrodes, the oligonucleotides having a sequence complementary to a first portion of the sequence of said nucleic acid, the contacting taking place under conditions effective to allow hybridization of the oligonucleotides on the substrate with said nucleic acid;

(b) contacting said nucleic acid bound to the substrate with an aggregate probe comprising nanoparticles having oligonucleotides attached thereto, at least one of the types of oligonucleotides on the aggregate probe comprising a sequence complementary to the sequence of a second portion of said nucleic acid, the nanoparticles of the aggregate probe being made of a material which can conduct electricity, the contacting taking place under conditions effective to allow hybridization of the oligonucleotides on the aggregate probe with the nucleic acid; and

(c) detecting a change in conductivity.

417. (Currently amended) [A method of detecting a nucleic acid wherein the method is performed on a substrate, the method comprising detecting the presence, quantity, or both, of the nucleic acid with an optical scanner] The method of Claim 416 wherein the substrate has a plurality of pairs of electrodes located on it in an array to allow for the detection of multiple portions of a single nucleic acid, the detection of multiple different nucleic acids, or both, each of the pairs of electrodes having a type of oligonucleotides attached to the substrate between them.

418. (Currently amended) [The method of Claim 417 wherein the device is a flatbed scanner] The method of Claim 416 wherein the nanoparticles are made of metal.

419. (Currently amended) [The method of Claim 417 wherein the scanner is linked to a computer loaded with software capable of calculating greyscale measurements, and the greyscale measurements are calculated, to provide a quantitative measure of the amount of nucleic acid detected] The method of Claim 416 wherein the nanoparticles are made of gold or silver.

420. (Currently amended) [The method of Claim 417 wherein the scanner is linked to a computer loaded with software capable of providing an image of the substrate, and a qualitative determination of the presence of the nucleic acid, the quantity of the nucleic acid, or both, is made] The method of Claim 416 wherein the substrate is contacted with silver stain to produce the change in conductivity.

421-422. (Previously cancelled)

423-424. (Currently cancelled)

425-432. (Previously cancelled)

433. (Currently amended) The method according to any one of claims 407, 412, 415 or 416, wherein the oligonucleotides are attached to the nanoparticles in a stepwise ageing process comprising (i) contacting the oligonucleotides with the nanoparticles in a first aqueous solution for a period of time sufficient to allow some of the oligonucleotides to bind to the nanoparticles; (ii) adding at least one salt to the aqueous solution to create a second aqueous solution; and (iii) contacting the oligonucleotides and nanoparticles in the second aqueous solution for an additional period of time to enable additional oligonucleotides to bind to the nanoparticles.

434. (Previously added) The method according to claim 433, wherein the salt solution has an ionic strength sufficient to overcome at least partially the electrostatic

attraction or repulsion of the oligonucleotides for the nanoparticles and the electrostatic repulsion of the oligonucleotides for each other.

435. (Previously added) The method of Claim 433 wherein the nanoparticles are metal nanoparticles or semiconductor nanoparticles.

436. (Previously added) The method of Claim 435 wherein the nanoparticles are gold nanoparticles.

437. (Previously added) The method of Claim 436 wherein the oligonucleotides include a moiety comprising a functional group which can bind to a nanoparticle.

438. (Currently amended) The method of Claim 433 wherein all of the salt is added to the [water] first aqueous solution in a single addition.

439. (Previously added) The method of Claim 433 wherein the salt is added gradually over time.

440. (Previously added) The method of Claim 433 wherein the salt is selected from the group consisting of sodium chloride, magnesium chloride, potassium chloride, ammonium chloride, sodium acetate, ammonium acetate, a combination of two or more of these salts, one of these salts in a phosphate buffer, and a combination of two or more of these salts in a phosphate buffer.

441. (Previously added) The method of Claim 440 wherein the salt is sodium chloride in a phosphate buffer.

442. (Currently amended) The method of any one of Claims 407, 412, 415 or 416 wherein the oligonucleotides present on surface of the nanoparticles at a surface density of at least 10 picomoles/cm².

443. (Previously added) The method of Claim 442 wherein the oligonucleotides are present on surface of the nanoparticles at a surface density of at least 15 picomoles/cm².

444. (Previously added) The method of Claim 443 wherein the oligonucleotides are present on surface of the nanoparticles at a surface density of from about 15 picomoles/cm² to about 40 picomoles/cm².

445. (New) A method of detecting a nucleic acid having at least two portions comprising:

(a) contacting the nucleic acid with a substrate having oligonucleotides attached thereto, the oligonucleotides being located between a pair of electrodes, the oligonucleotides having a sequence complementary to a first portion of the sequence of said nucleic acid, the contacting taking place under conditions effective to allow hybridization of the oligonucleotides on the substrate with said nucleic acid;

(b) contacting said nucleic acid bound to the substrate with a first type of nanoparticles, the nanoparticles being made of a material which can conduct electricity, the nanoparticles having one or more types of oligonucleotides attached thereto, at least one of the types of oligonucleotides having a sequence complementary to a second portion of the sequence of said nucleic acid, the contacting taking place under conditions effective to allow hybridization of the oligonucleotides on the nanoparticles with said nucleic acid, wherein the oligonucleotides are attached to the nanoparticles in a stepwise ageing process comprising (i) contacting the oligonucleotides with the nanoparticles in a first aqueous solution for a period of time sufficient to allow some of the oligonucleotides to bind to the nanoparticles; (ii) adding at least one salt to the aqueous solution to create a second aqueous solution; and (iii) contacting the oligonucleotides and nanoparticles in the second aqueous solution for an additional period of time to enable additional oligonucleotides to bind to the nanoparticles; and

(c) detecting a change in conductivity.

446. (New) The method of Claim 445 further comprising:

(d) contacting the first type of nanoparticles bound to the substrate with a second type of nanoparticles, the second type of nanoparticles being made of a material

which can conduct electricity, the second type of nanoparticles having one or more types of oligonucleotides attached thereto, at least one of the types of oligonucleotides on the second type of nanoparticles comprising a sequence complementary to the sequence of one of the types of oligonucleotides on the first type of nanoparticles, the contacting taking place under conditions effective to allow hybridization of the oligonucleotides on the first and second types of nanoparticles; and

(e) detecting the change in conductivity.

447. (Original) The method of Claim 446 wherein at least one of the types of oligonucleotides on the first type of nanoparticles has a sequence complementary to the sequence of at least one of the types of oligonucleotides on the second type of nanoparticles and the method further comprises:

(f) contacting the second type of nanoparticles bound to the substrate with the first type of nanoparticles, the contacting taking place under conditions effective to allow hybridization of the oligonucleotides on the first and second types of nanoparticles; and

(g) detecting the change in conductivity.

448. (New) The method of Claim 447 wherein step (d) or steps (d) and (f) are repeated one or more times and the change in conductivity is detected.

449. (New) The method of Claim 445 further comprising:

(d) contacting the first type of nanoparticles bound to the substrate with an aggregate probe comprising a second type of nanoparticles having one or more types of oligonucleotides attached thereto, the nanoparticles of the aggregate probe being made of a material which can conduct electricity, at least one of the types of oligonucleotides on the aggregate probe comprising a sequence complementary to the sequence of one of the types of oligonucleotides on the first type of nanoparticles, the contacting taking place under conditions effective to allow hybridization of the oligonucleotides on the aggregate probe with the oligonucleotides on the first type of nanoparticles;

(e) and detecting the change in conductivity.

450. (Twice amended) A method of detecting nucleic acid having at least two portions comprising:

(a) contacting a nucleic acid with a substrate having oligonucleotides attached thereto, the oligonucleotides being located between a pair of electrodes, the oligonucleotides having a sequence complementary to a first portion of the sequence of said nucleic acid, the contacting taking place under conditions effective to allow hybridization of the oligonucleotides on the substrate with said nucleic acid;

(b) contacting said nucleic acid bound to the substrate with an aggregate probe comprising nanoparticles having oligonucleotides attached thereto, at least one of the types of oligonucleotides on the aggregate probe comprising nanoparticles comprise a sequence complementary to the sequence of a second portion of said nucleic acid, the nanoparticles of the aggregate probe being made of a material which can conduct electricity, the contacting taking place under conditions effective to allow hybridization of the oligonucleotides on the aggregate probe with the nucleic acid, wherein the oligonucleotides are attached to the nanoparticles in a stepwise ageing process comprising

- (i) contacting the oligonucleotides with the nanoparticles in a first aqueous solution for a period of time sufficient to allow some of the oligonucleotides to bind to the nanoparticles;
- (ii) adding at least one salt to the aqueous solution to create a second aqueous solution; and
- (iii) contacting the oligonucleotides and nanoparticles in the second aqueous solution for an additional period of time to enable additional oligonucleotides to bind to the nanoparticles; and

(c) detecting a change in conductivity.

451. (New) The method of any one of Claims 445 or 450 wherein the substrate has a plurality of pairs of electrodes located on it in an array to allow for the detection of multiple portions of a single nucleic acid, the detection of multiple different nucleic acids, or both, each of the pairs of electrodes having a type of oligonucleotides attached to the substrate between them.

452. (New) The method of any one of Claims 445 or 450 wherein the nanoparticles are made of metal.

453. (New) The method of any one of Claims 445 or 450 wherein the nanoparticles are made of gold or silver.

454. (New) The method of any one of Claims 445 or 450 wherein the substrate is contacted with silver stain to produce the change in conductivity.

455. (New) The method of any one of Claims 445 or 450 wherein the salt solution has an ionic strength sufficient to overcome at least partially the electrostatic attraction or repulsion of the oligonucleotides for the nanoparticles and the electrostatic repulsion of the oligonucleotides for each other.

456. (New) The method of any one of Claims 445 or 450 wherein the nanoparticles are metal nanoparticles or semiconductor nanoparticles.

457. (New) The method of Claim 456 wherein the nanoparticles are gold nanoparticles.

458. (New) The method of Claim 457 wherein the oligonucleotides include a moiety comprising a functional group which can bind to a nanoparticle.

459. (New) The method of any one of Claims 445 or 450 wherein all of the salt is added to the first aqueous solution in a single addition.

460. (New) The method of any one of Claims 445 or 450 wherein the salt is added gradually over time.

461. (New) The method of any one of Claims 445 or 450 wherein the salt is selected from the group consisting of sodium chloride, magnesium chloride, potassium chloride, ammonium chloride, sodium acetate, ammonium acetate, a combination of two or more of these salts, one of these salts in a phosphate buffer, and a combination of two or more these salts in a phosphate buffer.

462. (New) The method of Claim 461 wherein the salt is sodium chloride in a phosphate buffer.

463. (New) The method of any one of Claims 445 or 450 wherein the oligonucleotides present on surface of the nanoparticles at a surface density of at least 10 picomoles/cm².

464. (New) The method of Claim 463 wherein the oligonucleotides are present on surface of the nanoparticles at a surface density of at least 15 picomoles/cm².

465. (New) The method of Claim 464 wherein the oligonucleotides are present on surface of the nanoparticles at a surface density of from about 15 picomoles/cm² to about 40 picomoles/cm².

466. (New) The method of any one of Claims 445 or 450 wherein at least some of the oligonucleotides on the nanoparticles comprise at least one type of recognition oligonucleotides, each type of recognition oligonucleotides comprising a spacer portion and a recognition portion, the spacer portion being designed so that it is bound to the nanoparticles, the recognition portion having a sequence complementary to at least a portion of a sequence of a selected type of binding oligonucleotides.

467. (New) The method of Claim 466 wherein the spacer portion has a moiety covalently bound to it, the moiety comprising a functional group through which the spacer portion is bound to the nanoparticles.

468. (New) The method of Claim 466 wherein the spacer portion comprises at least about 10 nucleotides.

469. (New) The method of Claim 468 wherein the spacer portion comprises from about 10 to about 30 nucleotides.

470. (New) The method of Claim 466 wherein the bases of the nucleotides of the spacer portion are all adenines, all thymines, all cytosines, all uracils or all guanines.

471. (New) The method of any one of Claims 445 or 450 wherein at least some the oligonucleotides bound to the nanoparticles comprise at least one type of recognition oligonucleotides, each type of recognition oligonucleotides comprising a sequence complementary to at least one portion of a sequence of a selected type of binding oligonucleotides; and a type of diluent oligonucleotides.

472. (New) The method of Claim 471 wherein each type of recognition oligonucleotides comprises a spacer portion and a recognition portion, the spacer portion being designed so that it is bound to the nanoparticles, the recognition portion having a sequence complementary to at least one portion of a sequence of a selected type of binding oligonucleotides.

473. (New) The method of Claim 472 wherein the spacer portion has a moiety covalently bound to it, the moiety comprising a functional group through which the spacer portion is bound to the nanoparticles.

474. (New) The method of Claim 472 wherein the spacer portion comprises at least about 10 nucleotides.

475. (New) The method of Claim 474 wherein the spacer portion comprises from about 10 to about 30 nucleotides.

476. (New) The method of Claim 472 wherein the bases of the nucleotides of the spacer portion are all adenines, all thymines, all cytosines, all uracils or all guanines.

477. (New) The method of Claim 472 wherein the diluent oligonucleotides contain about the same number of nucleotides as are contained in the spacer portions of the recognition oligonucleotides.

478. (New) The method of Claim 477 wherein the sequence of the diluent oligonucleotides is the same as that of the spacer portions of the recognition oligonucleotides.

479. (New) The method of any one of Claims 445 or 450 wherein the oligonucleotides and nanoparticles are contacted in first aqueous solution for about 12 to about 24 hours.

480. (New) The method of any one of Claims 445 or 450 wherein salt is added to the aqueous solution to form the second aqueous solution which is buffered at pH 7.0 and which contains about 0.1 M NaCl.

481. (New) The method of any one of Claims 445 or 450 wherein the oligonucleotides and nanoparticles are contacted in the second aqueous solution for an additional 40 hours to increase the density of oligonucleotides bound to the nanoparticles.

482. (New) A kit for detecting one or more target nucleic acids in a sample, the target nucleic acid having at least two portions, the kit comprising:

(i) a substrate having attached thereto one or more pairs of electrodes and at least one type of oligonucleotides attached to the substrate between the electrodes, each type of oligonucleotides having a sequence that is complementary to at least a first portion of a sequence of a specific target nucleic acid; and

(ii) nanoparticles having one or more types of oligonucleotides attached thereto, at least one of the types of oligonucleotides having a sequence complementary to a second portion of the sequence of said target nucleic acid, wherein the oligonucleotides are attached to the nanoparticles in a stepwise ageing process comprising (i) contacting the oligonucleotides with the nanoparticles in a first aqueous solution for a period of time sufficient to allow some of the oligonucleotides to bind to the nanoparticles; (ii) adding at least one salt to the aqueous solution to create a second aqueous solution; and (iii) contacting the oligonucleotides and nanoparticles in the second aqueous solution for an additional period of time to enable additional oligonucleotides to bind to the nanoparticles.

483. (New) The kit of Claim 482 wherein the substrate has a plurality of pairs of electrodes attached to it in an array to allow for the detection of multiple portions of a single nucleic acid, the detection of multiple different nucleic acids, or both.